including January 28, 2002. The Assistant Commissioner is hereby authorized to charge the required $400 extension fee to Deposit Account No. 23-1703. Any other fees which may be due in connection with this Petition may likewise be charged.

REMARKS

Applicant acknowledges the Examiner's tacit withdrawal of all prior objections to the claims and all prior indefiniteness and enablement rejections.

Claims 1-12, 14 and 15 are pending in the application, and have been finally rejected in the present Office Action on the grounds that "Applicant's amendment necessitated the new ground(s) of rejection presented in this Office Action." However, Applicant notes that, in each rejection set forth in the present Action, the Examiner states that the grounds of rejection are "explained in the previous Office action." Thus, there are no **new** grounds of rejection whatsoever, either necessitated or not by Applicant's amendment; in fact, as evidenced by the Examiner's withdrawal of a number of prior rejections, Applicant's previous amendments can, if anything, only be said to have reduced and simplified the outstanding issues. The grounds for finality of the rejections are inappropriate and the finality should be withdrawn.

In any event, Applicant emphatically objects to the Examiner's continued use of impermissible hindsight to combine a series of prior art documents which might nominally be said to collectively describe the claimed invention. A common thread running throughout the Examiner's stated grounds of rejection is the allegation that instant SEQ ID NO: 3 is identical to SEQ ID NO: 3 disclosed in more than one of the cited references. Whether or not the Examiner's allegation is true, this teaching, by itself or combined with other cited teachings, cannot be held to render obvious the instant invention. Applicant is not claiming the molecule set forth as SEQ ID NO: 3 or any other polypeptide for that matter. The instant invention is directed to patentable systems for the expression of the molecule set forth in SEQ ID NO: 3 and molecules homologous thereto. Inappropriately, the Examiner is treating a nucleic acid encoding a given polypeptide as patentably indistinct from a nucleic acid containing said coding region as a component and constituting in total a vector capable of expressing the encoded polypeptide.

However, the Examiner has again failed to recognize the key features of the instant invention and has again failed to appreciate the significant unpredictability of the art of heterologous protein expression which, at the filing date of the

present application, would have rendered this alleged, and, in any event, nominal, combination nonobvious to a person of skill in the art. Moreover, none of the cited prior art references teach that hBSSL can successfully be expressed in biologically active form in *Pichia pastoris*, or, for that matter, any yeast whatsoever.

Contrary to what the Examiner may imply, this is not a question of simply trying to move from successful expression in *Saccharomyces cerevisiae* to expression in *Pichia pastoris*. The Examiner is in fact trying to make the much longer leap from expression in *E. coli* and mammalian cells to expression in *Pichia pastoris*. In doing so, the Examiner fails to recognize at all the unpredictability in the art and the need for the recombinantly expressed hBSSL to be properly processed (i.e., glycosylated, folded etc.) and biologically active.

Applicant previously pointed out the unpredictability in the art of recombinant yeast expression and the fact that the Examiner's obviousness rejections were based on hindsight. However, the Examiner has reiterated these arguments and stated that "a reconstruction based upon hindsight" is permissible "so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from

applicant's disclosure...." Applicant does not argue with this statement. However, Applicant must again point out 1) that the Examiner's selection of "knowledge... within the level of ordinary skill at the time..." is insufficient to have allowed one to arrive at the present invention and 2) that the Examiner has failed to recognize additional knowledge at the time that would have acted against the teachings cited by the Examiner and, if anything, would have discouraged pursuit of the instant invention. Thus, the hindsight used by the Examiner does not conform with his own definition of what is permissible.

The Examiner also opines that "[T]here is no substantial evidence provided by the Applicant that the unpredictability in the art and the equally significant discouraging results known to those working in the field is such that one of ordinary skill in the art would not have even consider combining the teachings cited." In response to these comments and order to more fully address the issue of unpredictability in the art, Applicant brings to the Examiner's attention the publication of Ratner, Bio/Technology **7**: 1129-1133 (1989); a copy of this reference is provided herewith.

Applicant particularly wishes to direct the Examiner's attention to the following passages in the Ratner reference:

1) In the first paragraph on page 1129, it is disclosed that optimal expression of a given protein depends on a number of considerations, among them whether the protein is expressed intracellularly or secreted into the medium; the need for appropriate glycosylation, refolding and other posttranslational modifications; and the possibility of antigenicity.

2) In the second paragraph on page 1129, both *S. cerevisiae* and *P. pastoris* are mentioned in connection with attendant uncertainty as to whether products therefrom are appropriately glycosylated.

3) In both the third paragraph on page 1129 and the first paragraph on page 1133 is addressed the idea that each evaluation of a system must be done on a case-by-case basis and that each system is protein specific.

4) In the beginning of the third paragraph on page 1133, it is stated that: "Genzyme's reluctance to consider a license was compounded by questions about the biological activity of the protein grown with *P. pastoris*--particularly t-PA. 'Their leading example--t-PA--is not biologically active,'...."

It is clear from the Ratner reference that, at the filing date of the instant application, there was significant unpredictability in the art of successful heterologous

expression in yeast. The uncertainty and unpredictability is further enhanced when one adds to this the necessity for expression of a large, heavily glycosylated human protein in correctly processed and biologically active form and at high levels.

Another factor contributing to the inability of a person skilled in the art, even at the present time, to predict or appreciate that a given protein, in this case hBSSL, will be expressed in a biologically active form stems from the known fact that some recombinantly expressed proteins are subject to endogenous proteolytic degradation. It is for this reason that protease-deficient mutant host strains are generally used for recombinant expression of heterologous proteins. Such "sick" strains may not be suitable, however, for expression of large protein genes such as the 3-kB gene required to encode the 722-amino-acid hBSSL protein. Until the present invention, then, there could have been no certainty for those in the field that hBSSL expressed in *P. pastoris* cells would not be subjected to endogenous cellular proteolytic degradation. This is yet another factor that would have worked against motivation to make or even try to make the instant invention.

Bearing all of the above in mind, Applicant now addresses below the specific prior art rejections leveled by the Examiner.

Claims 1-12, 14 and 15 stand rejected as constituting obviousness-type double patenting over claims 1-6, 9, 10, 12 and 14 of U.S. Patent No. 5,827,683 to Bläckberg et al. in view of Martinez et al. Furthermore, claims 1, 2, 4-12, 14 and 15 stand rejected under 35 USC §103(a) as obvious over U.S. Patent No. 5,200,183 to Tang et al. in view of U.S. patent No. 4,808,537 to Stroman et al. Finally, claims 3 and 14 stand rejected under 35 USC §103(a) as obvious over the Tang patent in view of Martinez.

The Bläckberg patent describes at length the preparation of variant forms of wild-type hBSSL. Although the patent teaches and claims expression vectors housing the variant hBSSL-encoding nucleic acids and generically discloses expression of such variant hBSSL's in recombinant systems, it only exemplifies mammalian cells, *E. coli* (as a basis for comparison) and transgenic animals. None of the examples teach successful yeast expression in any yeast species, let alone expression in *Pichia pastoris*. Thus, there is no evidence or even suggestion provided that biologically active and correctly processed hBSSL can be successful expressed in *Pichia pastoris* in particular, let alone yeast generically.

The Tang patent teaches the cloning, sequencing and expression of the cDNA encoding full-length hBSSL protein. The

Examiner asserts that Tang et al. teach expression of the lipase in yeast. Although the specification describes various expression systems including yeast, this is merely in a generic or prophetic sense, and no actual expression is described or exemplified. With respect to "yeast as host," the inventors refer to a general methods paper in "Methods in Enzymology" and state that "[L]ike *E. coli*, yeast host cells **may** express a foreign gene either in the cytosol or as a secreted protein." [Emphasis added.] Such a generalized teaching does not by any means even provide much hope or expectation, let alone ensure, that biologically active human proteins in general, let alone BSSL in particular, can be successfully produced in such a heterologous yeast host. Tang et al. do not unambiguously teach that full-length hBSSL can be recombinantly expressed in yeast to yield correctly-processed, biologically-active protein.

What both prior art hBSSL patents do teach is that hBSSL is a large, glycosylated protein. hBSSL has many *O*-glycosylation sites which will be glycosylated to varying sizes and complexities due to various factors. The *N*- and *O*-linked carbohydrates in proteins in mammalian cells are quite different from each other and very different from those in yeast, both in carbohydrate content and structure. Further, oligosaccharide structures attached to proteins are also different.

Glycosylation plays an important role in the proper folding of recombinant proteins, as the amino acids are assembled during protein synthesis in the host cells. In the case of hBSSL, a heavily glycosylated protein, one of skill in the art at the time of the present invention would not have been able to predict, and, in fact, would not have expected, that a *Pichia pastoris* recombinantly-expressed hBSSL protein would be appropriately glycosylated and enzymatically active. It cannot even be concluded, based on the present knowledge in the field, that a given gene would be expressed in a biologically useful way in a heterologous expression system, and the uncertainty was even greater at the time of filing of the instant application.

The secondary reference of Martinez et al. teaches only that *Pichia pastoris* is suitable for high-level expression of some heterologous proteins. This does not at all convey that heterologous protein genes can be generally expressed in this system nor, more importantly, that any expressed proteins would exhibit the biological activities of the native proteins.

The secondary Stroman patent teaches various expression vectors comprising one of a number of different carbon-source (e.g., methanol, glucose, ethanol, fructose, glycerol, galactose and the like)-inducible regulatory promoters, not just the alcohol oxidase promoter, for use in various yeast hosts (i.e.,

not just *Pichia pastoris*; see column 15). The teaching of Stroman adds nothing over that of Martinez and thus, when combined with Tang, does not make up for the deficiencies of Martinez. Stroman teaches only that *Pichia pastoris*, among many other yeasts including *S. cerevisiae*, can be used to express some heterologous proteins in an inducible manner. This does not at all imply or even suggest that heterologous protein genes can be expressed in this system, nor, still more importantly, that any expressed protein will retain its native biological activity.

As Applicant pointed out in his previous response, the yeast host of choice at the time of the instant invention was, and still remains, *S. cerevisiae*; the Examiner's assertions are based on the benefit of impermissible hindsight. For example, if, as the Examiner suggests, it was obvious that *P. pastoris* could successfully express biologically active and correctly processed hBSSL at high levels, then, prior to the instant invention, it would surely have also been obvious that *S. cerevisiae* could be used to express high levels of biologically active and correctly processed recombinant hBSSL, since the Stroman patent teaches that their expression vectors can, inter alia, be used with *S. cerevisiae* (see last paragraph of column

20 to top of column 21). This was not the case, however (see page 18 of the instant application).

Applicant found that the expression of native full-length human hBSSL in *S. cerevisiae* was very difficult. He found that the expression level was too low to be quantified; indeed, it could only be detected on Western gels. Furthermore, the native signal sequence was not cleaved, thus inevitably resulting in intracellular sequestration of expressed protein. The fact that successful expression was not achieved in the "gold-standard" system emphasizes the unpredictability associated with recombinant expression per se and the lack of expectation engendered by the knowledge in the art. Furthermore, the fact highlights the deficiencies of the Stroman teaching as prior art. And, again, since the well-known "reliable" system was insufficient, it certainly cannot be held that the use of a *Pichia pastoris* system would have been *prima facie* obvious at the time of filing of the instant application.

The above difficulties with *S. cerevisiae* notwithstanding, Applicant, unexpectedly, was able ultimately to demonstrate efficient expression in *P. pastoris* with both a native signal sequence and with an *S. cerevisiae* signal sequence.

In summary, the successful expression in a heterologous system of a protein with its native biological activity is the

result of number of factors including (a) proper choice of signal peptide; (b) optimal glycosylation of the recombinant protein produced to render a proper folding of the protein; (c) proper growth conditions; and (d) purification protocols designed to obtain maximum yields with complete retention of biological activity of the expression product. The instant invention, unexpectedly, embodies all of the required factors and thus overcomes the many deficiencies in the teachings of the cited prior art. Furthermore, the success of the instant invention goes against other prior knowledge, not cited by the Examiner, in the field. The instant invention must thus be considered novel and nonobvious over the cited teachings, and the rejection should be withdrawn. Reconsideration and allowance of the pending claims is respectfully requested.

The Assistant Commissioner is hereby authorized to charge any fees which may be due for any reason to Deposit Account No. 23-1703.

Dated: January 28, 2002

Respectfully submitted,

Paul Diamond
Reg. No. 48,532

Applicants' Agent
Customer Number 007470
(212) 819-8200

Agent's Direct Line:
(212) 819-8425

Enclosure (Ratner, Bio/Technology 7: 1129-1133 (1989))